Role of α-Dystroglycan as a Schwann Cell Receptor for *Mycobacterium leprae*

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α-Dystroglycan (α-DG) is a component of the dystroglycan complex, which is involved in early development and morphogenesis and in the pathogenesis of muscular dystrophies. Here, α-DG was shown to serve as a Schwann cell receptor for *Mycobacterium leprae*, the causative organism of leprosy. *Mycobacterium leprae* specifically bound to α-DG only in the presence of the G domain of the α2 chain of laminin-2. Native α-DG competitively inhibited the laminin-2–mediated *M. leprae* binding to primary Schwann cells. Thus, *M. leprae* may use linkage between the extracellular matrix and cytoskeleton through laminin-2 and α-DG for its interaction with Schwann cells.

Pathogenic bacteria are adapted to exploit a variety of host cell functions, and host cell receptors mostly serve as the initial target for bacterial interaction with a specific cell type (*1, 2*). However, not much is known about the bacterial receptors in the nervous system and how bacteria interfere with these neuronal cell receptor–associated functions. *Mycobacterium leprae*, the causative organism of leprosy, is an intracellular pathogen that invades the Schwann cell of the peripheral nervous system (*3*). During infection, *M. leprae* causes significant damage to peripheral nerves leaving patients with disabilities and deformities (*4*). Although antibiotic therapy is an effective cure of leprosy, it does not reverse the nerve function loss in these patients (*5*). Understanding the mechanisms of *M. leprae*–Schwann cell interaction may yield new therapeutic strategies for the prevention of nerve damage.

Dystroglycan (DG), a component of the dystrophin-glycoprotein complex, is a laminin receptor encoded by a single gene and cleaved by posttranslational processing into two proteins, peripheral membrane α-DG and transmembrane β-DG (*6*). Whereas α-DG interacts with laminin-2 in the basal lamina, β-DG appears to bind to dystrophin-containing cytoskeletal proteins in muscles and peripheral nerves (*7*). DG is involved in agrin- and laminin-induced acetylcholine receptor clustering at neuromuscular junctions (*8*), morphogenesis (*9*), early development (*10*), and the pathogenesis of muscular dystrophies (*6*, *7*). The loss or a defect of laminin-2–α-DG interaction causes certain types of muscular dystrophy and peripheral neuropathy (*11, 12*). We recently showed that laminin-2 in the basal lamina of the Schwann cell–axon unit serves as an initial target for *M. leprae* interaction with peripheral nerves (*13*). Laminin-2, which comprises α2, β1, and γ1 chains (*14*), anchors to Schwann cells through laminin receptors (*15*). α-DG serves as a receptor on the Schwann cell that interacts with laminin-2 in the basal lamina surrounding the Schwann cell-axon unit (*7, 16*).

To determine the role of α-DG in the *M. leprae* interaction with Schwann cells, we first examined the binding of *M. leprae* (*17*) to native α-DG purified from peripheral nerves (*18*) in the presence or the absence of recombinant fragments of the laminin-α2 chain (*19*) (Fig. 1, A and B). In a solid-phase assay (*20*), *M. leprae* bound to immobilized α-DG only in the presence of the COOH-terminal fragment of the laminin-α2 chain (rLNα2G; Fig. 1A). *Mycobacterium leprae* also bound to muscle α-DG only in the presence of rLN-α2G (Fig. 1A). *Mycobacterium leprae* binding to α-DG of both peripheral nerve and muscle was increased by >95% with a concentration of 10 μg/ml (0.1 μg per well) of rLNα2G. Even higher concentrations (100 μg/ml) of the NH_2-terminal r(VI-IVb)′ fragment of laminin-α2 chain or the G domain of the laminin-α1 chain (rLNα1G) had no effect on *M. leprae* binding to α-DG (110 ± 24 and 130 ± 31 bacteria per 0.25 mm^2, respectively), suggesting that the G domain of the laminin-α2 chain specifically mediated *M. leprae* binding to α-DG. Thus, LNα2G has two binding sites, one for *M. leprae* and the other for α-DG, and the G domain forms a bridge between *M. leprae* and α-DG. Additionally, the activity of merosin/α2 laminins (a mixture of laminin-2 and laminin-4) on *M. leprae*–α-DG interaction yielded results similar to those with rLNα2G at equal molar ratio (1010 ± 110 and 1290 ± 161 bacteria per 0.25 mm^2, respectively).

Comparison of rLNα2G-mediated *M. leprae* binding to native (Fig. 1B) versus fusion proteins of α-DG (Fig. 2, B and C) showed that the bacteria strongly bound only to the native α-DG in a concentration-dependent manner (Fig. 2A). Because the native confor-

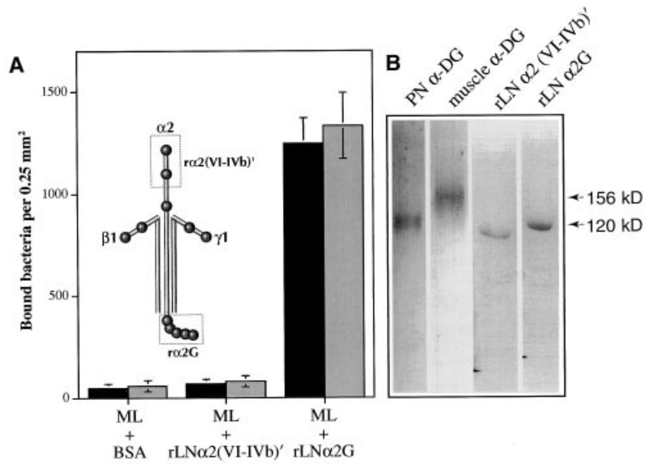

Fig. 1. *Mycobacterium leprae* binding to immobilized α-DG is mediated by the G domain of the laminin-α2 chain. (**A**) Quantification of *M. leprae* binding to immobilized native α-DG purified from peripheral nerves (PN-αDG) (black bars) and skeletal muscles (muscle-αDG) (gray bars) in the presence of recombinant fragments of the laminin-α2 chain. *Mycobacterium leprae* (5×10^8) was preincubated with rLNα2G or rLNα2(VI-IVb)′ (10 μg/ml), and the mixtures were overlaid onto immobilized α-DG (50 μg/ml). The number of adherent *M. leprae* (ML) within a 0.25 mm^2 grid area of each well was quantified after 60 min of incubation, and the data were expressed as the mean ± SD values from five to six wells. Three additional experiments gave similar results. The inset is a laminin-2 molecule showing the location of the NH_2-terminal (VI-IVb)′ fragment and the COOH-terminal G domain of the α2 chain. (**B**) Coomassie blue–stained SDS-PAGE gel showing the purified native α-DG preparations and recombinant laminin-α2 chain fragments used in the study: peripheral nerve α-DG (120 kD), muscle α-DG (156 kD), rLN-α2 (VI-IVb)′ (116 kD), and rLN-α2G (120 kD).

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mation of α-DG seems unnecessary for the laminin interaction, as denatured DG also binds laminins (*7*) and *M. leprae*+rLNα2G (*21*), glycosylation is the most likely post-translational modification that contributes to α-DG's interaction with *M. leprae* through the LN-α2G domain. Because the rLNα2G-mediated *M. leprae* binding to α-DG was sensitive to periodate (Fig. 2D), the carbohydrate moieties of α-DG are likely important for rLNα2G-mediated *M. leprae* interactions. Although α-DGs are differentially glycosylated in different tissues (for example, peripheral nerve and muscle) of the same species, which contributes to different molecular sizes (*22*) (Fig. 1B), glycosylation of α-DG in a given tissue (for example, peripheral nerve) is almost identical in mouse, rabbit, cow, and human (*6, 22–24*). Further characterization of the rLNα2G-mediated *M. leprae*–α-DG interaction showed that this binding is completely abolished by EDTA, indicating the crucial role of calcium for the interaction of the G domain with α-DG. Moreover, the lack of inhibitory effects of heparin on rLNα2G-mediated *M. leprae* binding to α-DG (Fig. 2E) suggests that the heparin binding site of the G domain of the laminin-2 molecule is different from the α-DG binding site. Thus, the G domain is the α-DG binding site of the laminin-2 molecule, and this interaction is dependent on calcium and is largely mediated by the carbohydrate moieties of α-DG. This may be of significant physiological relevance in muscular dystrophies because the loss of laminin-2 interaction with α-DG is critical for the disease pathogenesis (*6, 7*).

To determine whether peripheral nerve α-DG serves as a Schwann cell receptor for *M. leprae*, we purified Schwann cells from rat sciatic nerves (*25*). These primary Schwann cells strongly expressed α-DG but showed almost no deposition of LNα2G in early cultures (Fig. 3C) (*21*); they were also devoid of certain laminin receptors, for example, the integrin β4 subunit (*25*). Because *M. leprae* binds to

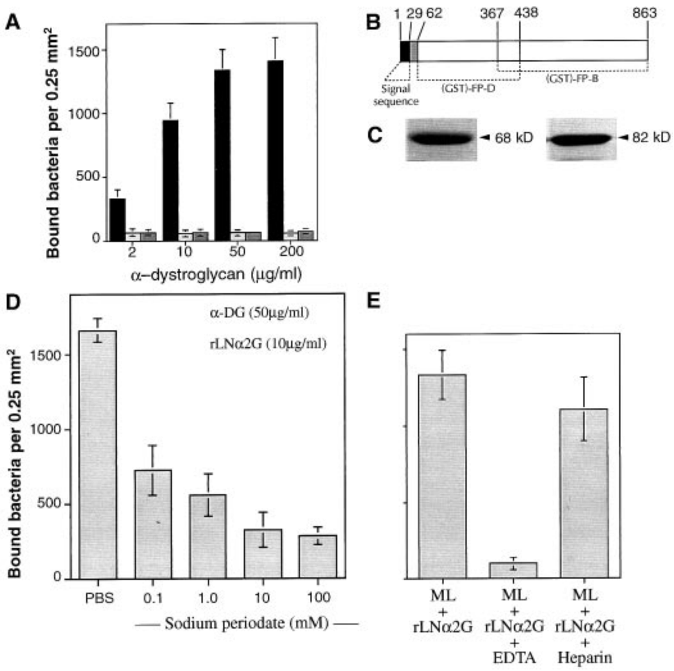

Fig. 2. Characteristics of rLN-α2G–mediated *M. leprae* binding to α-DG. (**A**) *Mycobacterium leprae* adherence to increasing concentrations of native peripheral nerve α-DG (black bars) and fusion proteins FP-B (light gray bars) and FP-D (dark gray bars) of human DG in the presence of human rLN-α2G (10 μg/ml). *Mycobacterium leprae* binding to α-DG was expressed by subtracting the values of *M. leprae*+rLN-α2G binding from *M. leprae*+BSA binding. Data shown are the means ± SE of triplicates of *M. leprae* adherence at each concentration of α-DG from a representative experiment. Two additional experiments gave similar results. (**B**) Diagram showing the fusion proteins of α-DG used in this study. Two segments corresponding to the α-DG core protein, except amino acid residues 30 to 61, were expressed as glutathione S-transferase (GST) fusion proteins (*6*). Fusion protein–D (FP-D) contains amino acid residues 62 to 438 and fusion protein–B (FP-B) contains amino acid residues 367 to 863. The predicted signal sequence contains amino acid residues 1 to 29. (**C**) Coomassie blue–stained SDS-PAGE gel showing the purified FP-D (left) and FP-B (right). (**D**) Effect of sodium periodate treatment on rLN-α2G–mediated *M. leprae* binding to α-DG. Increasing concentrations of sodium periodate were added to immobilized α-DG before the incubation with *M. leprae*+rLN-α2G mixture. Bound bacteria were determined by acid-fast labeling and expressed as mean ± SD. (**E**) rLN-α2G–mediated *M. leprae* binding to α-DG (50 μg/ml) in the presence of 10 mM EDTA and heparin (1 mg/ml). Data shown are mean ± SE from three experiments.

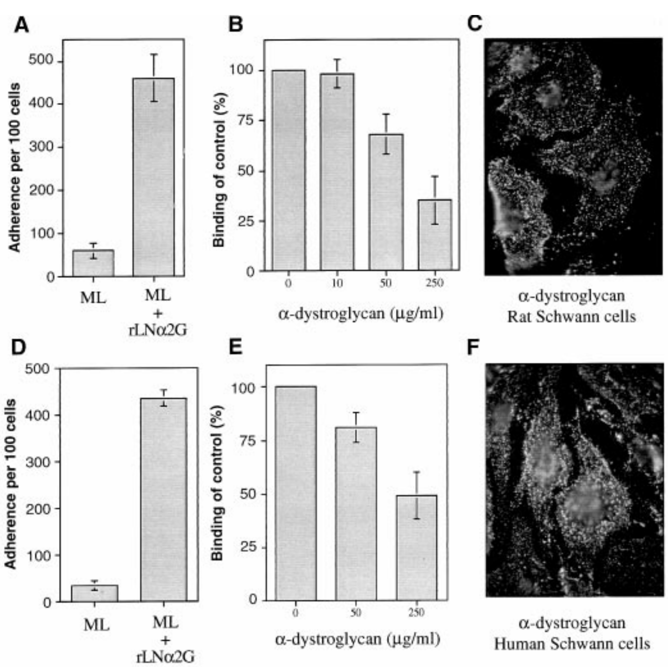

Fig. 3. α-DG is involved in rLN-α2G–mediated *M. leprae* adherence to rat Schwann cells. (**A**) *Mycobacterium leprae* adherence to primary Schwann cells in the presence or the absence of rLN-α2G. Purified Schwann cells grown for 3 days without forskolin were inoculated with *M. leprae*, which were preincubated with either rLN-α2G or BSA (10 μg/ml). The number of *M. leprae*–bound Schwann cells were expressed per 100 cells, and data were presented as mean ± SD from three experiments. (**B**) Competitive inhibition of rLN-α2G–mediated *M. leprae* binding to primary rat Schwann cells by native peripheral nerve α-DG. rLN-α2G–coated *M. leprae* were preincubated with increasing concentrations of purified α-DG and the mixture was added onto Schwann cells. Cell-bound *M. leprae* were detected by acid-fast labeling and values are presented as percent binding of control (mean ± SD) obtained from three experiments. Net rLN-α2G–mediated *M. leprae* adherence to Schwann cells [(*M. leprae*+rLN-α2G) – (*M. leprae*+BSA)] in the absence of α-DG was considered as 100% binding. (**C**) α-DG expression on primary Schwann cells purified from rat sciatic nerve as shown by immunofluorescence labeling by mAb IIH6C4 to α-DG. (**D**) *Mycobacterium leprae* adherence to human Schwann cells in the presence or absence of rLN-α2G. Experiments were performed in similar conditions as in (A). (**E**) Competitive inhibition of rLN-α2G–mediated *M. leprae* binding to human Schwann cells by native α-DG. Experiments were performed in similar conditions as in (B). (**F**) α-DG expression on immortalized human Schwann cells as detected by immunofluorescence with mAb IIH6C4.

rLNα2G with high affinity (*13*) and exogenous rLNα2G efficiently mediated *M. leprae* adherence to primary Schwann cells (Fig. 3A) (*26*), this model system enabled us to study the involvement of α-DG in G domain–mediated *M. leprae*–Schwann cell interactions independent of the influence of other regions of the laminin-2 molecule. Using this system in competition assays (*27*), we found that rLNα2G-mediated *M. leprae* binding to Schwann cells was inhibited by preincubation of the *M. leprae*+rLNα2G complex with native α-DG in solution (median inhibitory concentration, IC_{50} = 160 μg/ml) (Fig. 3B). Fusion proteins of α-DG had no inhibitory effect on *M. leprae* binding to Schwann cells (*21*). Thus, it is likely that the carbohydrate moieties of α-DG are involved in G domain–mediated *M. leprae* interaction with Schwann cells.

To investigate whether the above findings of α-DG on primary rat Schwann cells holds equally true for humans, we used immortalized human Schwann cells for the binding and invasion assays. Monoclonal antibody (mAb) IIH6C4 (*6*), which is specific for α-DG, strongly reacted with human Schwann cells (Fig. 3F), and the pattern of α-DG expression on the dorsal surface of the live cells was in the form of microclusters as found for primary rat Schwann cells (Fig. 3, C and F). Although *M. leprae* alone showed almost no binding to human Schwann cells, preincubation of *M. leprae* with a 10 μg/ml concentration of rLNα2G resulted in a >90% increase of cellular adherence (Fig. 3D). This binding was inhibited by preincubation of the *M. leprae*+rLNα2G complex with native α-DG (IC_{50} = 250 μg/ml) (Fig. 3E). The low inhibitory effect of α-DG on *M. leprae*+rLNα2G binding to human Schwann cells, as compared with primary rat Schwann cells (Fig. 3B), may be due to the increased expression of other molecules (or secretory products) on the transformed human Schwann cells that mask the inhibitory effect of native α-DG. Nevertheless, the data suggest the involvement of α-DG in LNα2G-mediated *M. leprae* adherence in both rat and human Schwann cells. However, our data do not exclude other mechanisms of *M. leprae* adherence of Schwann cells because purified α-DG was unable to compete 100% for rLNα2G-mediated *M. leprae* adherence, suggesting the participation of other Schwann cell laminin receptors.

To substantiate further the involvement of α-DG as a Schwann cell receptor for *M. leprae*, we examined the effect of rLNα2G-coated *M. leprae* on the distribution of α-DG receptors on live primary Schwann cells (*28*). α-DG is a mobile receptor on muscle cells and forms clusters when it interacts with matrix proteins (*28*). In control primary Schwann cell cultures, α-DG labeling, as determined by mAb IIH6C4, was equally distributed as microclusters on the dorsal surface of live Schwann cells (immunolabeled as individual dots; Fig. 4A). Microclusters (each dot) on unstimulated Schwann cells may represent single α-DG molecule, as suggested previously for muscle cells (*28*). When Schwann cells were challenged with rLNα2G-coated *M. leprae* (after removing free rLNα2G), α-DG labeling on most of the Schwann cells appeared as aggregates or macroclusters with different sizes and shapes over the entire dorsal cell surface (Fig. 4, B and C). Bacterial challenge caused a dramatic decrease of microclusters and increased the number of macroclusters (compare Fig. 4A with Fig. 4, B and C), which suggests that macroclusters were derived from microclusters. C and D of Fig. 4 demonstrate the colocalization of α-DG receptor clusters and *M. leprae* on the same Schwann cell. Because *M. leprae* alone failed to induce cluster formation (*21*), the clustering of α-DG on Schwann cells appeared to be contributed by the *M. leprae*–bound LNα2G. These data strongly suggest that the α-DG participates in the LNα2G-mediated *M. leprae* interaction with Schwann cells.

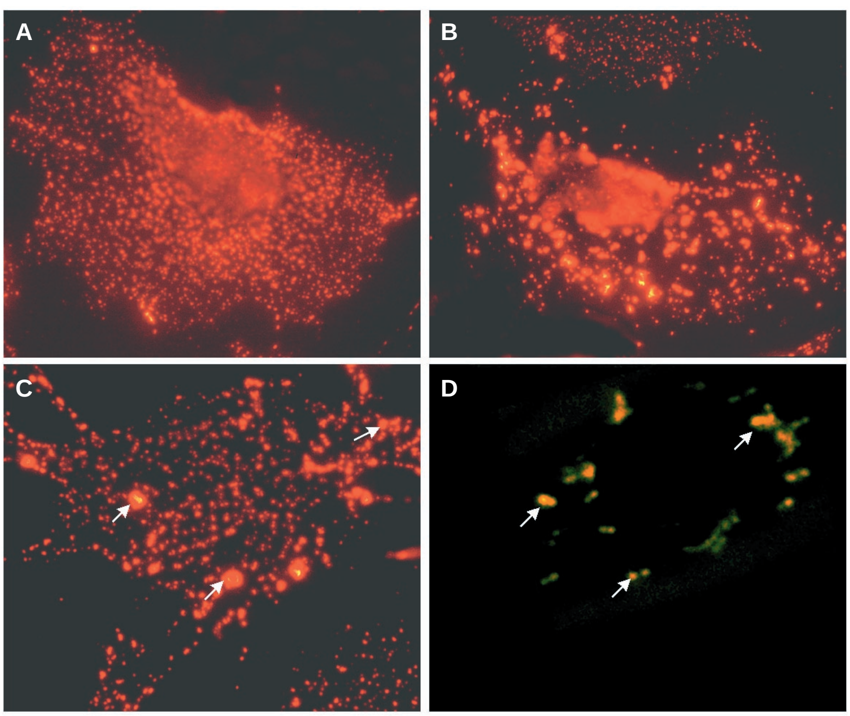

Fig. 4. α-DG receptor clustering on Schwann cells induced by rLN-α2G–coated *M. leprae*. (**A**) Immunofluorescence of α-DG showing its distribution on the dorsal surface of live primary Schwann cells before bacterial challenge. The areas corresponding to the nuclei of cells are out of focus. (**B** and **C**) Representative examples showing different forms of macroclusters of α-DG 3 hours after bacterial challenge. Comparison of (A) with (B) and (C) reveals that rLN-α2G–coated *M. leprae* induced an extensive aggregation of α-DG receptors on the dorsal surface of Schwann cells. (**C** and **D**) Colocalization of α-DG receptor clusters and *M. leprae* on the same Schwann cell as detected by immunodouble labeling with mAbs to α-DG (IIH6C4) (C) and *M. leprae*–specific PGL-1 (D). The arrows mark the α-DG clustering site approximately corresponding with Schwann cell-bound *M. leprae*. All labeling was viewed with a 100× oil immersion objective.

Pathogenic bacteria are particularly adapted to exploit host cell functions (*1, 2*). In peripheral nerves, α-DG appears to link extracellular laminin-2 to the intracellular cytoskeleton through β-DG and associated proteins (*6–8*, *16*). In addition to playing a structural role, this system also regulates host cell functions (*6, 7*). Present data suggest that *M. leprae* is adapted to exploit this matrix-cytoskeleton link of the peripheral nervous system for its own benefit; *M. leprae* adhere and possibly invade Schwann cells and subsequently interfere with neural cell functions associated with this system.

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17. *Mycobacterium leprae* was purified from armadillos and provided by P. J. Brennan (Colorado State University, Fort Collins, CO). Each isolate was tested for acid-fast labeling and *M. leprae*–specific phenolic glycolipid-1 (PGL-1) reactivity with a auramine-rhodamine Bacto TB Fluorescent Stain Kit (Difco, Detroit, MI) and mAb to native PGL-1, respectively, before the assays.
18. The bovine peripheral nerve α-DG was purified as described [(*16*); H. Yamada, T. Shimizu, T. Tanaka, K. P. Campbell, K. Matsumura, *FEBS Lett.* **352**, 49 (1994); J. M. Ervasti, S. D. Kahl, K. P. Campbell, *J. Biol. Chem.* **266**, 9161 (1991)]. The rabbit skeletal muscle α-DG was purified by using the same method but with KCl-washed heavy microsomes of rabbit skeletal muscle as a starting material. α-DG fusion proteins B and D (FP-B and FP-D) were prepared as described (*6*).
19. Recombinant (r) LN-α2G, rLN-α2(VI-IVb)′, and rLN-α1G fragments were prepared with a baculovirus expression system as previously described [(*13*); P. D. Yurchenco, U. Sung, M. D. Ward, Y. Yamada, J. J. O'Rear, *J. Biol. Chem.* **268**, 8356 (1993)]. Human merosin (laminin-2 and laminin-4) was a gift from M. Paulsson. The purity of DG preparations and recombinant fragments of laminins were analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with antibodies specific for each fraction as described (*13, 18, 19*).
20. *Mycobacterium leprae* binding to α-DG was determined with a solid-phase bacterial adherence assay (*13*) by using immobilized native α-DG purified from peripheral nerves or skeletal muscles, or recombinant α-DG. Terasaki plates were coated overnight with α-DGs (50 μg/ml, 0.5 μg per well) or bovine serum albumin (BSA) as a negative control. *Mycobacterium leprae* (5×10^8 bacteria/ml) suspension was preincubated with rLNα2G or LNα2(VI-IVb)′ (10 μg/ml, 0.1 μg per well) or BSA for 1 hour at 37°C. After blocking the nonspecific binding with BSA, 10 μl of the *M. leprae* mixture was added to each well and incubated for 1 hour at 37°C. Unbound bacteria were removed by washing with DPBS and wells were fixed with 2.5% glutaraldehyde (Sigma). Adherent *M. leprae* was detected by acid-fast labeling, counted, and expressed as described (*13*). The effect of heparin and EDTA on rLNα2G-mediated *M. leprae* binding to α-DG was determined similarly by incubating the bacterial mixture with 10 mM EDTA or heparin (1 mg/ml). The effect of periodate treatment was evaluated by preincubation of increasing concentrations of sodium periodate with native α-DG before the addition of *M. leprae*+rLNα2G. Periodate and EDTA treatment did not detach the α-DG from wells because no difference was found in antibody activity to α-DG before and after treatment as detected by enzyme-linked immunosorbent assay with polyclonal antibodies to α-DG.
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25. Schwann cells were isolated from neonatal rat sciatic nerve, purified, and amplified as described [S. Einheber, T. A. Milner, F. Giancotti, J. L. Salzer, *J. Cell Biol.* **123**, 1223 (1993)]. Human Schwann cells were purified and immortalized as described [J. L. Rutkowski, J. S. Rhim, K. W. C. Peden, G. I. Tennekoon, in *Neoplastic Transformation in Human Cell Systems in Vitro*, J. S. Rhim and A. Dripschillo, Eds. (Humana, Totowa, NJ, 1991), pp. 343–346]. Schwann cells were plated onto poly-L-lysine–coated eight-well Lab-Tek chamber slides (Nunc) or 12-mm cover slips and cultured without forskolin to prevent the deposition of laminin-2. These primary rat Schwann cells and human Schwann cells were found to be 100% pure as determined by antibody to S-100 antigen.
26. Primary rat Schwann cells and immortalized human Schwann cells were used for both adherence and invasion assays because they are devoid of LNα-2G. The *M. leprae* adherence assay to Schwann cells was previously described (*13*).
27. For competitive inhibition assays, rLNα2G-coated *M. leprae* were preincubated with increasing concentrations of native α-DG for 3 hours at 37°C, after which the mixture was added onto Schwann cells, and the adherence assays were performed as described (*13*). The number of acid-fast–labeled bacteria were quantified, and values were presented as the mean percent binding of controls. The net rLNα2G-mediated *M. leprae* adherence to Schwann cells was considered as 100%.
28. Light microscopy and immunofluorescence of Schwann cells were performed as described (*13*). Characterization of mAb IIH6C4 to α-DG and affinity-purified rabbit polyclonal antibody (pAb) to human rLN-α2G were described previously (*7, 13*). The mAb F47-21 to native PGL-1 was a gift from A. H. J. Kolk (Royal Tropical Institute, Amsterdam). The pAb to S-100 was from Sigma. α-DG detection and clustering studies were performed as previously reported [M. W. Cohen, C. Jacobson, P. D. Yurchenco, G. E. Morris, S. Carbonetto, *J. Cell Biol.* **136**, 1047 (1997)]. For bacterial-induced α-DG clustering, *M. leprae* was preincubated with rLN-α2G for 1 hour at 37°C, and the mixture was centrifuged and the pellet was resuspended in phosphate-buffered saline (PBS) to avoid the contact of free rLN-α2G with Schwann cells. These rLN-α2G–coated *M. leprae* were added onto primary Schwann cells as described in adhesion assays. Cultures were then stained live and fixed with 2.5% glutaraldehyde before processing for fluorescence microscopy. In live Schwann cells, α-DG labeling is restricted to the dorsal surface because IIH6C4 immunoglobulin M mAb is unable to reach the ventral cell surface due to its large size. Colocalization of α-DG and *M. leprae* was performed by double immunofluorescence with mAb IIH6 and mAb to *M. leprae* PGL-1.
29. We thank P. J. Brennan for providing *M. leprae* through a National Institute of Allergy and Infectious Disease/NIH contract, M. Zschack for graphics, and S. Terlow for *M. leprae* preparations. We also thank E. Tuomanen for the initial support and encouragement for this study. Supported by grants from the United Nations Development Programme/World Bank/World Health Organization Special Program for Research in Tropical Diseases and NIH (A.R., V.A.F., J.L.S., and P.Y.). H.Y. was supported by an American Heart Association fellowship and by the Mizutani Foundation. K.P.C. is an HHMI investigator.

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Identification of α-Dystroglycan as a Receptor for Lymphocytic Choriomeningitis Virus and Lassa Fever Virus

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A peripheral membrane protein that is interactive with lymphocytic choriomeningitis virus (LCMV) was purified from cells permissive to infection. Tryptic peptides from this protein were determined to be α-dystroglycan (α-DG). Several strains of LCMV and other arenaviruses, including Lassa fever virus (LFV), Oliveros, and Mobala, bound to purified α-DG protein. Soluble α-DG blocked both LCMV and LFV infection. Cells bearing a null mutation of the gene encoding DG were resistant to LCMV infection, and reconstitution of DG expression in null mutant cells restored susceptibility to LCMV infection. Thus, α-DG is a cellular receptor for both LCMV and LFV.

Arenaviruses consist of several causative agents of fatal human hemorrhagic fevers (*1, 2*). Among these pathogens, LFV causes an estimated 250,000 cases and more than 5000 deaths annually (*1, 3*). LCMV, the prototype arenavirus, has been studied primarily in its natural rodent host as a model of viral immunology and pathogenesis (*4*).

To initiate infection, the LCMV glycoprotein GP-1 anchors the virus to the cell surface through a proteinaceous receptor (*5, 6*), which by a virus overlay protein blot assay (VOPBA) (*7*) was identified as a single high molecular weight glycoprotein (*5*). The presence of the receptor protein correlated directly with a cell's susceptibility to LCMV attachment and infection (Fig. 1). Its broad migration pattern on SDS-polyacrylamide gels is likely to reflect the heterogeneity in cell type–specific posttranslational modifications (*5*). In addition to murine cells, a broad range of rodent and primate cells express the same protein (*5*) (Fig.